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A<sup>1</sup>

(Amended) **Figures 2A-2C.** Determination of *mda-7* transcription by nuclear run-on, *mda-7* mRNA by Northern blotting and RT-PCR and a comparison of the AU-rich sequences found in the 3'-UTR of several mRNAs. (Figure 2A) Nuclear run-on assays using nuclei isolated from Control or 24 h after treatment with IFN- $\beta$ , MEZ or IFN- $\beta$  + MEZ, the same concentrations as indicated in Fig. 1. GAPDH was used as an internal control. In vitro transcription assays were performed as previously described (Jiang et al., 1993). (Figure 2B) *Mda-7* message expression detected by Northern blotting and RT-PCR followed by Southern blotting. Total RNA from control (Figure 2C) HO-1 cells and cells treated with IFN- $\beta$  (I), MEZ (M) or IFN- $\beta$  +MEZ (I+M) were analyzed by Northern blotting or RT-PCR/Southern using radiolabeled *mda-7* cDNA as a probe as previously described (Jiang et al., 1993, 1995a; Kang et al., 1998a). GAPDH was used as an internal loading control. (C) Several cytokine genes and proto-oncogenes that contain the AUUUA consensus sequence in their 3'-UTRs. Abbreviations: Hu = human; *mda-7* = melanoma differentiation associated gene-7 (Jiang et al., 1995a);  $\alpha$ -IFN = alpha interferon (Goeddel et al., 1981); GM-CSF = granulocyte-monocyte colony stimulating factor (Wong et al., 1985); TNF = tumor necrosis factor (Nedwin et al., 1985); cFos = fos proto-oncogene (van Straaten et al., 1983). (SEQ ID NOS:9-13).

Please amend the paragraph on page 57, line 25 through page 58, line 5. A clean version of the amended paragraph follows:

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(Amended) **Isolation and cloning of the *mda-7* promoter.** A human placental genomic library (Stratagene) was screened using the *mda-7* cDNA (18) labeled by random priming (Life Technologies, Inc.)



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CONT.

using  $\alpha$ -<sup>32</sup>P[dCTP]. Three 1 phage clones were identified and isolated to homogeneity. An anti-sense primer, 5'-CGTCCCAGCCGTGGAAGTCAT-3' (SEQ ID NO:2) corresponding to the region 40-50 bp from the 5' terminal end of the mda-7 cDNA was used with the T3 or T7 primer in a polymerase chain reaction to amplify the region upstream of the mda-7 cDNA from the three 1 phage clones. The proof reading polymerase, Tth polymerase (Clontech) was employed for this purpose. One of the 1 phage clones yielded a 2.2 Kbp amplification product that was cloned into pBluescript and sequenced (ABI sequencing).

Please amend the paragraph on page 59, line 20 through page 60, line 12. A clean version of the amended paragraph follows:

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B1

(Amended) **Electrophoretic Mobility Shift Assay (EMSA)**. Nuclear extracts were prepared as described (25). Briefly, binding reactions were performed in 10 or 20 ml reaction mixtures containing 1-3 mg of nuclear extracts from control or differentiation inducer treated HO-1 cells. The binding buffer contained 12 mM HEPES (pH 7.9), 5 mM MgCl<sub>2</sub>, 60 mM KCl, 0.6 mM EDTA, 0.5 mM dithiothreitol, 1 mg of poly (dI-dC), 10% glycerol. The region corresponding to the putative AP-1 and C/EBP binding sites present between NdeI and NheI restriction enzyme sites was PCR amplified using flanking primers, 5'-AGGCTGGATTTG GCTTGTGAC-3' (Sense) (SEQ ID NO:3) and 5'-CTGTTTAATCCAGCACTTCCC-3' (Antisense) (SEQ ID NO:4). The PCR product was column purified (Qiagen), end labeled with  $\gamma$ -<sup>32</sup>P [ATP] and 1500 cpm of double stranded DNA were used per binding reaction. Binding reactions were performed at RT for 30 min. Reactions were then loaded onto a 4% polyacrylamide gel and electrophoresed at 4° C at 100 V in 0.25X Tris-borate-EDTA as described (26,27). Competition and supershift reactions were identical to those described above, except a 10-100 fold excess of